Efficient sample preparation in immuno-matrix-assisted laser desorption/ionization mass spectrometry using acoustic trapping

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Acoustic trapping of minute bead amounts against fluid flow allows for easy automation of multiple assay steps, using a convenient aspirate/dispense format. Here, a method based on acoustic trapping that allows sample preparation for immuno-matrix-assisted laser desorption/ionization mass spectrometry using only half a million 2.8 μm antibody covered beads is presented. The acoustic trapping is done in 200×2000 μm^2 glass capillaries and provides highly efficient binding and washing conditions, as shown by complete removal of detergents and sample processing times of 5-10 min. The versatility of the method is demonstrated using an antibody against Angiotensin I (Ang I), a peptide hormone involved in hypotension. Using this model system, the acoustic trapping was efficient in enriching Angiotensin at 400 pM spiked in plasma samples. © *2013 American Institute of Physics*. [http://dx.doi.org/10.1063/1.4798473]

INTRODUCTION

The use of mass spectrometry (MS) in clinical diagnostics can provide new means for patient stratification and personalized medicine.[1,2] This development started to gain momentum with the introduction of MS-based proteomic pattern diagnostics using surface enhanced laser desorption/ionization (SELDI) analysis.[3,4] Despite the ensuing controversies regarding reproducibility and sensitivity of the method,[5–9] the SELDI approach highlighted the fact that MS could play an important a role in clinical diagnostics, and that the combination of immuno-affinity extraction and MS was a promising way forward.[10–13] Although an MS-based immunoassay cannot currently match the detection levels of the most sensitive optical alternatives an MS-readout enables; unequivocal identification of antigens, discovery of cross-reacting species, and pinpointing structural variants of an antigen that a conventional ELISA-based assays cannot distinguish.[14,15]

For MS-based diagnostics to compete with current antibody-based clinical diagnostics, a number of criteria have to be matched. These include both pre-analytical, e.g., sample storage, preparation and study design, as well as analytical, e.g., quantization, sensitivity, robustness, and reproducibility. Many of these challenges for MS-based clinical proteomics have been addressed, as evident by the success of selected reaction monitoring or multiple reaction monitoring (SRM/MRM) MS-based technology platforms where multiplexed assays for a wide range of proteins have been demonstrated.[16,17] The sensitivity of MRM based methods of unfractionated samples is typically in the μg/ml range,[18] in order to facilitate MS-based analysis of lower abundant biomarkers the use of immuno-affinity and isotope dilution has been suggested,[19–24] a strategy that can allow for detection of analyte concentrations at ng/ml levels. While this is a lower sensitivity than that of a well-developed ELISA, the MS approach offers other advantages such as multiplexing, throughput and structural information of the analyte bound to the antibody.

A key for efficient implementation of MS-based diagnostics is the ability to process samples from large patient cohorts. Currently, there are two main approaches to MS-based

clinical proteomics that uses antibody affinity purification, SRM/MRM liquid chromatography (LC) MS based methods that provides high sensitivity at low throughput, and immuno matrix-assisted laser desorption/ionization (iMALDI) MS based methods that provides a higher throughput at lower sensitivity.[20] The high throughput of MALDI MS as compared to LC-MS based approaches is especially promising for clinical proteomics on large patient cohorts. With improved affinity protocols and technology development, it may be possible to make immuno-MALDI MS a valuable technique in clinical settings, similarly to how MALDI MS is now routinely used for bacteria identification.[25]

A wide variety of iMALDI MS approaches have been presented in the literature.[11,26–44] Most standard affinity-methods are based on capture on magnetic beads with immobilized antibodies and rely on detergents for solubilization and reduction of unspecific binding. We have recently presented an iMALDI MS platform using a strategy of detergent-free immunocapture on porous silicon[44] as an alternative to bead based assays. The use of detergents in MALDI MS generates an intense background that reduces ionization efficiency and yields additional peaks that can mask the desired signal. After immunocapture, the target antigen is eluted from the beads causing a dilution. Therefore, a solid-phase extraction (SPE) step is often implemented to re-concentrate the antigen after acid-shock elution from the antibody. Although, if reverse phase (RP) beads are used for the SPE the presence of hydrophobic detergents will compete with the antigen for binding to the RP beads thereby significantly lowering sensitivity or even making analysis impossible. In standard magnetic bead based sample preparation extensive washing and transfers of the beads to new containers is employed to get rid of the detergents, but the drawback is that the removal of detergents causes extensive losses of beads as they will stick to surfaces of pipettes and containers.

In order to facilitate the use of detergents in the assay protocols while still being able to take advantage of RP-SPE an alternative iMALDI method based on acoustic trapping of antibody-beads in a capillary followed by miniaturized RP-SPE before MALDI MS analysis was developed. The RP-SPE sample preparation was done on an ISET-chip, as previously described. This allows for re-concentration of analyte in a minuscule elution volume and as the ISET also serves as a MALDI target sample transfers are avoided. The use of acoustics in microfluidic assays has many applications,[45–47] including the use of surface acoustic standing waves for nebulization in mass-spectrometry.[48–50] Acoustic trapping in glass capillaries was recently demonstrated as a robust and low-cost way of realizing non-contact cell or bead handling.[51,52] Combining miniaturized ultrasonic transducer technology with a rectangular cross-section glass capillary enables non-contact retention of minute cell-/bead-populations in a flow by means of ultrasonic standing wave forces.[52] This allows for easy automation of multiple assay steps, using an ordinary aspirate/dispense format.

In addition to trapping the acoustic standing wave also inherently generates acoustic streaming. The acoustic streaming has previously been shown to improve mixing and enzymatic turnover in microchannels.[53,54] The fact that the beads are retained in non-contact mode as a thin layer,[51] in combination with the efficient mixing, allows very efficient washing and binding conditions. This enables removal of detergents and assures a low background, both prerequisites for high-sensitivity and -specificity assays. It is shown that the presented method can be used to minimize the amount of antibody covered beads used per sample and drastically reduce the incubation time. The method reduces the cost and time for analysis, as well as allows the use of detergents to minimize unspecific binding. The acoustic microbead retention technology is here reported as a powerful tool for sample preparation in iMALDI analysis in an assay for Angiotensin I (Ang I), a peptide hormone involved in hypotension.

MATERIAL AND METHODS

Chemicals

Unless otherwise specified all chemicals were purchased from Sigma-Aldrich Co (St. Louis, MO, US) and used without further purification. Rabbit polyclonal antibody Angiotensin I (Ab-Ang I) was purchased from Abcam (Cambridge, UK). 2.8 μm magnetic beads (Dynabeads

M-270 Carboxylic Acid or Dynabeads Protein A/G) and Poros R2 20 μm beads were purchased from Life Technologies (NY, US). HPLC grade water was used for preparation of all aqueous suspensions. Isotope labeled Angiotensin I, human 13C, and 15N was purchased from BioNordica (Stockholm, Sweden).

Sample preparation

Bovine serum albumin (BSA) was digested with trypsin (Promega, Madison, WI, USA) in a 1:100 ratio (enzyme:protein) for 90 min in 37 °C. The digested peptide mixture was diluted 100 times with 10 mM phosphate buffered saline (PBS), pH 7.4 and frozen to stop the digestion. A stock solution of 1 μM BSA was used to prepare standard samples by dilution and spiking with antigen.

Human plasma was diluted 1:10 with 10 mM PBS containing protease inhibitor cocktail, P8340 from Sigma-Aldrich. Co. (St. Louis, MO), spiking with Angiotensin I to specified concentrations was done immediately before experiments.

Trapping capillaries

To facilitate acoustic trapping a previously described setup[51] based on borosilicate capillaries with a rectangular cross-section was used (VitroCom, NJ, USA), The inner dimensions of the capillary were $200 \times 2000\ \mu m^2$ and the wall thickness was 100 μm. With a water-filled channel, this geometry has a cross-sectional resonance at 4 MHz (resembling a standing wave with $\lambda/2 = 200\ \mu m$), Figure 1. Actuating the cross-sectional resonance using a miniaturized 4 MHz transducer at 17 V_{pp} creates a spatially localized acoustic field that enables non-contact retention of particles against fluid flow.

As has been previously shown,[51] the particles are retained in an approximately 10-20 μm thick layer in the center of the channel. In the experiments, the acoustic trap was saturated with beads resulting in a reproducible number of Ab-beads to be levitated in a thin layer and retained against fluid flows around 100 μl/min for washing.

The trapping capillary was connected to a syringe pump and used in aspirate/dispense-mode (from the open end of the capillary) thereby allowing easy integration with other standard lab equipment. Beads and samples were aspirated from a test tube and after washing, the beads and liquid were directly dispensed into an ISET nanovial for analysis, Figure 2. A linear-motor neMESYS syringe pump (Cetoni, Korbußen, Germany) was used for precise control of aspiration/dispense operations. The total volume of the trapping capillary was 20 μl and experimental parameters such as flow rate and fraction collection volume were as specified in the Results and Discussion section.

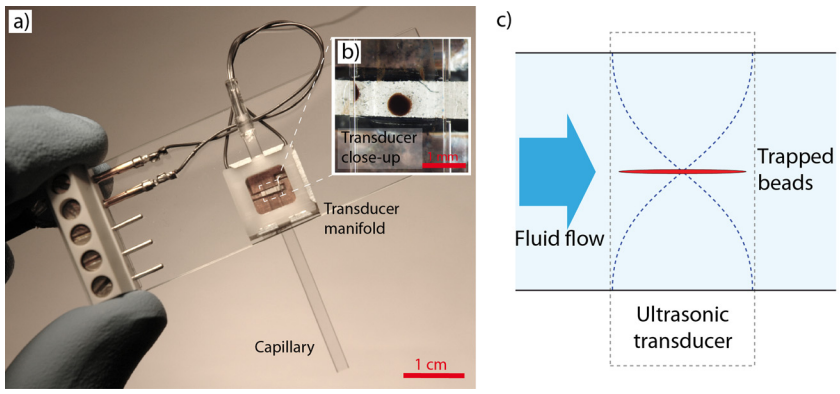

FIG. 1. Rectangular cross-section $200 \times 2000\ \mu m^2$ trapping capillary and transducer set-up (a) used like a pipette in aspirate/dispense-mode, allowing trapping of a controlled amount of beads (b) above the millimeter sized ultrasonic transducer. The schematic side view (c) shows how the beads are retained in a thin layer by ultrasonic forces. These forces allow retention against fluid flow for buffer exchange and washing purposes.

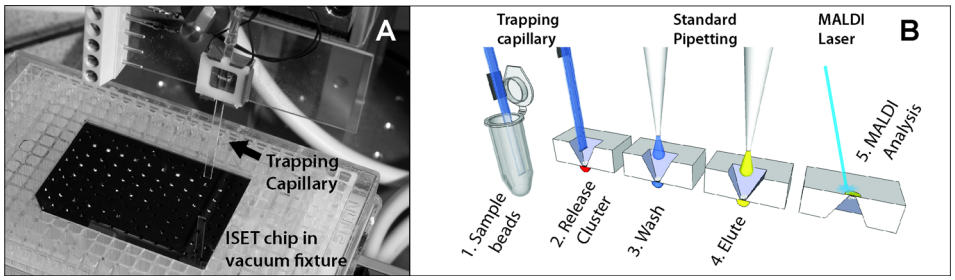

FIG. 2. (a) Photo of the acoustic trapping capillary over the ISET RP-SPE chip in its vacuum fixture (made in a 384 microtitre plate). (b) Schematic work-flow of immunocapture using the proposed acoustic trapping iMALDI method: (1) Antibody beads are trapped and sample solution is aspirated and incubated in the rectangular acoustic trapping capillary (blue). (2) The washed beads with bound antigen are released on top of an ISET nanovial packed with SPE beads and the antigen is transferred from the Ab-beads to the SPE. (3) Buffer salts are washed away and antigen displaced from the antibody beads and captured on the SPE beads. (4) The antigen is eluted from the SPE beads onto the analysis zone on the backside of the ISET using matrix. (5) The ISET is inserted up-side down in the MALDI MS instrument and analyzed.

The number of trapped beads in a saturated cluster was determined by manual counting in a hemocytometer (Bürker counting camber). The saturated cluster was formed by aspirating an excess and subsequently reversing the flow to remove the excess beads. In total, five clusters of 2.8 μm Ab-beads were formed and released into a volume of 40 μl for subsequent counting.

Acoustic trapping-based immunocapture protocol

In the presented method, antibody beads were aspirated and trapped in the capillary. To allow optimal comparison, identical beads were used in the acoustic trapping protocol as in the standard magnetic based assay, although in the acoustic case, the magnetic properties were not required or used. After an initial wash of the beads in the acoustic trap, the sample was aspirated and the antigen allowed to bind to the antibody-beads. The antigen capture was done in trap either by aspirating a fixed sample volume or by using slow (10 μl/min) aspirate/dispense cycles of the sample solution during the incubation. After antigen capture, the beads were washed with 100 μl PBS system fluid (running buffer) and released into an ISET nanovial prefilled with SPE beads. The acoustic trapping protocol was as follows:

1. Aspirate bead suspension and saturate the trap with beads.
2. Aspiration of the sample.
3. Wash of the beads with bound antigen by dispensing 100 μl of PBS that is used as system fluid.
4. Release of the cluster in 10 μl PBS into a ISET nanovial.

In order to re-concentrate and desalt the antigen, the previously described[44,55–58] integrated selective enrichment target (ISET) was used. Here, Poros R2 20 μm beads (Carlsbad, CA, US) were used as RP-SPE media to purify and concentrate the antigen. The ISET target was placed in a vacuum fixture and the vacuum was supplied by a vacuum pump (Vacuubrand GMBH, Wertheim, Germany), controlled and gauged with a valve (Qiagen vacuum regulator, product NO. 19530) inserted between the vacuum pump and the fixture. The ISET RP-SPE protocol was as follows:

1. Prior to application of the eluted sample each perforated nanovial was filled with approximately 100 nl R2 beads in 60% ACN/0.1%TFA and washed with 2.5 μl 60% ACN/0.1%TFA followed by 0.1% TFA, at maximum vacuum.
2. The antibody beads with captured antigen was dropped on-top of individual ISET positions filled with RP-SPE media and the sample solution was drawn through the packed bed by applying a vacuum (−5 mmHg). Thereafter, addition of 5 μl acetic acid elution was likewise drawn through to allow transfer of the antigen from the Ab-beads to the SPE-beads.
3. The positions were then washed with 2 × 3 μl 0.1% TFA twice, under high vacuum (−10 mmHg).

4. Prior to elution with matrix, the ISET was removed from the vacuum fixture and the backside of the target dried with a tissue.
5. Elution of the analytes onto the backside of the ISET was performed with $2 \times 0.3\,\mu$l, 60% ACN/0.1%TFA containing 1 mg/ml of cyano-4-hydroxy-cinnamic acid (CHCA). The elution was done at a low vacuum (−2 mmHg).

For the final MALDI MS analysis, the ISET chip was turned up-side down, i.e., with the matrix spots facing upwards into a MALDI target having a milled recession accepting the chip.

Manual immunocapture protocol

The angiotensin I antibody was immobilized onto Dynabeads according to the manufacturer protocol.[59] The following protocol was used for manual affinity capture and elution with the magnetic beads:

1. 10 μl solution of antibody coated magnetic beads in PBS ($\sim 2 \times 10^6$ beads) was added to 10 μl sample in a 0.6 ml tube (maximum recovery tube, Axygen, Union City, CA, US), and allowed to bind for 1 h at room temperature in a shaker.
2. The tube was placed on a magnet to pellet the magnetic beads on the side of the tube. The supernatant was aspirated, and the beads were washed with 20 μl PBS. This washing procedure was repeated three times. In order to minimize background from detergents, the magnetic beads were transferred to a new tube after the first wash cycle.
3. After the final wash the magnetic beads were re-suspended in 10 μl 5% Acetic acid and incubated for 5 min in order to elute the captured target.
4. The eluate (10 μl of 5% Acetic acid) was directly transferred to the ISET and subjected to RP-SPE sample preparation, just like the samples from the acoustic trapping.

Mass spectrometry

Samples were analyzed with MALDI-MS on a M@ldi LR (Waters/Micromass, Milford, MA, USA) and/or a MALDI Orbitrap XL (Thermo Scientific, Waltham, MA, USA) instrument. Prior to data acquisition, the MALDI settings were optimized to provide the best possible resolution and mass accuracy. For the MALDI TOF MS analysis, a spectrum of 100 summed laser shots was acquired for each sample spot and for MALDI Orbitrap analysis FT-MS 10 scans/spot were acquired. Resulting spectra from the Orbitrap were processed by XCALIBUR software v2.0.7 (Thermo Scientific, Waltham, MA, USA) or MASSLYNX 4.1 for the Waters instrument.

RESULTS AND DISCUSSION

Trapping protocol development

The use of ISET RP-SPE for re-concentration was applied due to the fact that the trapped bead cluster is very small, and direct deposition of the cluster onto the MALDI target was not possible as the minimum amount of liquid that has to be dispensed together with the cluster is too large (10-15 μl) to be accommodated on a MALDI standard spot position. After immunocapture enrichment of the antigen on the Ab-beads in the acoustic trap, the efficient antigen transfer to the RP-SPE phase in the ISET is important for minimizing sample losses. Two methods were investigated; elution of the antigen in the capillary followed by transfer of the eluate to the ISET and, transfer of the antigen-antibody beads to the ISET and elution in the ISET. It was found that release of the trapped antigen-antibody beads into the ISET nanovials pre-filled with RP-SPE media provided the highest sensitivity. This suggests that retaining the antigen on beads during the transfer avoids unnecessary absorptive losses and dilution of the sample.

The required washing volume is to a large extent determined by the dispersion in the capillary, as the washing volume needs to exceed the dispersion in the system. To determine a proper washing volume, the dispersion of an incubated peptide was measured with MALDI MS

and compared to the intensity of a spiked isotope of the same peptide. Note that no antibody capture was performed and that the spiked isotope was added for determination/quantitation of when the aspirated peptide was washed away. In brief, the capillary was filled with 20 μl peptide 2 (1 pmol/μl) this sample solution was incubated for 60 min in order to account for diffusion. After incubation 20 μl fractions were dispensed from the trapping capillary into a microplate. The fractions were analysed by taking 1 μl aliquots of each fraction and add 1 μl isotope standard peptide (+7 Da) at the same concentration as the originally aspirated sample (1 pmol/μl). The mixture was subsequently analyzed with MALDI MS after conventional dried droplet sample preparation. The spectra shown in Figure 3 revealed that the signal originating from the incubated peptide (peptide 2) disappeared between the second and the third fraction. As the first 20 μl fraction corresponds to the original aspirated sample volume this implies that a washing volume of 20 μl is sufficient to overcome detectable sample dispersion. The primary dispersion-mechanisms are diffusion (during incubation) and the parabolic flow profile (during sample aspiration). The diffusion distance for a typical protein ($D \sim 7 \times 10^{-11}$) under 60 min incubation is approximately 1.3 mm. In the used tubing (*tygone,* 0.298 mm *ID*) this corresponds to contaminating a sub-microliter volume. Loading of the sample creates a significant dispersion due to the parabolic flow profile. A plane Poiseuille flow model (regarding the capillary as two plates with 200 μm spacing) was used to model the magnitude. As the maximum flow rate in a plane Poiseuille flow is 3/2 times higher than the average flow rate a 3/2 times larger volume can be considered to be contaminated. Therefore, loading a 20 μl sample will contaminate an additional volume of 10 μl, due to the Poiseuille flow. The combined theoretical dispersion should thus only contaminate a volume that is less than 20 μl, as observed in the experiments. As diffusion plays a minor role in comparison to the parabolic flow profile, the dispersion depends more heavily on the volume of the loaded sample than the incubation time.

Washing efficiency

In addition to the detergents added to the assay-buffers magnetic beads are typically delivered in a small amount of Tween 20 or similar for prevention of aggregation. Even if no additional detergents are used for the assay this initial amount (<0.1%) is still a problem for iMALDI assays using a standard protocol, Figure 4 shows the results after the acoustic trapping

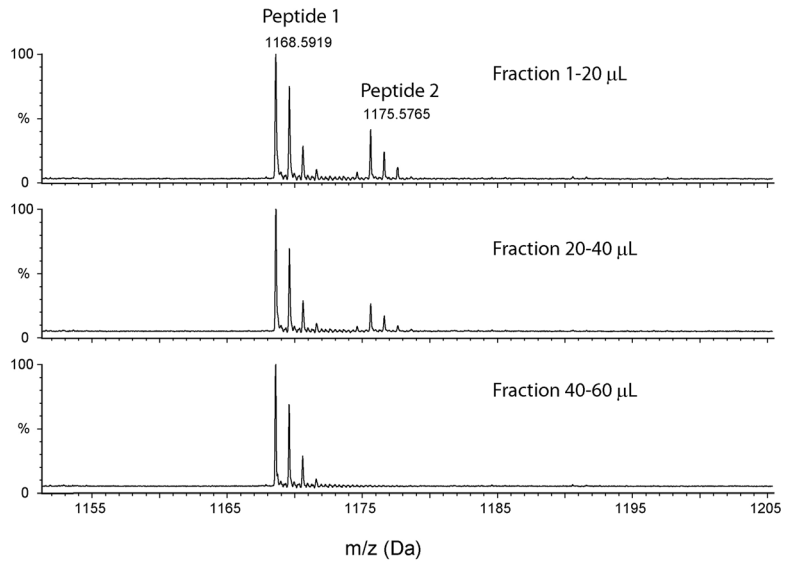

FIG. 3. Investigation of dispersion in the system: Peptide 2 at 1 pmol/μl was aspirated and incubated in the trapping capillary for 60 min. After collection of 20 μl fractions, the samples were mixed 1:1 with an isotope of the same peptide (peptide 1) and spotted on a MALDI target for analysis. Note that in the 3rd fraction (spectra) no more peptide 2 can be observed which implies that the levels of peptide 2 are at least 1000 times lower than that of peptide 1.

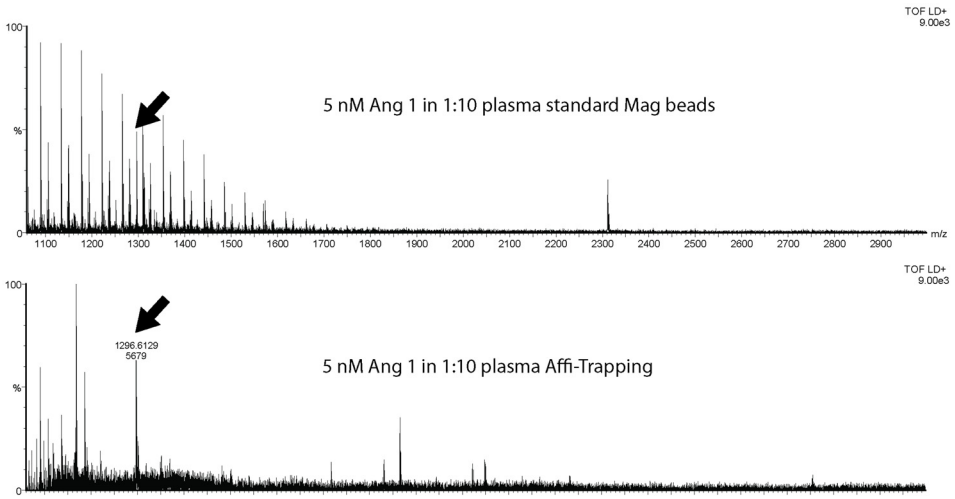

FIG. 4. Efficient wash of detergents. The lower spectrum results from an acoustic trapping iMALDI analysis of a 15 μl sample containing 5 nM Ang I in 1:10 diluted human plasma. The top spectra show the results of a standard assay of the same sample amount using the magnetic beads according to manufacturer specifications. Note the intense background ladder from the tween 20, which hampers analysis at low concentrations.

iMALDI, respectively, the standard sample preparation protocol. The best way to minimize the impact of detergents is to employ an extensive washing after Ab-coupling but unfortunately the removal of tween from the protocol will make the sample handling extremely difficult as the beads then will adhere to the walls of tubes and pipettes. Thus, careful visual inspection and sometimes extra pipetting steps were undertaken in order to minimize the bead losses during the standard manual assay, particularly during the transfer of the beads from the capture tube to the 2nd wash tube.

The ability to ensure efficient washing is important in order to remove detergents and reduce the non-specific background. This increases the final sensitivity, especially at low levels and thus the chance of observing true binding interactions in the final MS read-out. The ability to wash away a highly complex background with the acoustic trapping protocol was also verified by specific capture of angiotensin I at 1 nM from a 15 μl sample containing a 200 fold excess of background BSA peptides. After the acoustic iMALDI protocol, the unspecific background was washed away leaving the specific captured Ang I as the main observed peak, Figure 5. Contamination introduced by carry-over from the outer surface of the trapping capillary was avoided by applying a hydrophobic outer surface coating and a very short dip wash prior to deposition of the Ab-Antigen beads into the ISET for RP-SPE. Also by using a short methanol wash in-between samples memory effects were eliminated.

Incubation times

The initial experiments revealed that the incubation times, typically 1 h, used in the standard protocol could be reduced when using the microfluidic acoustic trapping system. For samples containing more than 1 nM Ang I, there was no difference in the final read-out when shortening the 1 h incubation times to 5 min by basically just aspirating and dispensing the sample at 10 μl/min flow rate. The decrease in incubation time can likely be attributed to, reduced diffusion distances in the microfluidic system, and the efficient mixing in the capillary owing to acoustic streaming. As shown previously, the acoustic trap generates four recirculating streaming vortexes in the vicinity of the transducer, which facilitates mixing in proximity of the trapped bead cluster,[60] Figure 6. Analogous, acoustic streaming has been previously demonstrated to allow increased enzymatic activity[53] and the technique has also been commercialized (Microsonic Systems, CA, US) for use in microtiter plate mixing.[54]

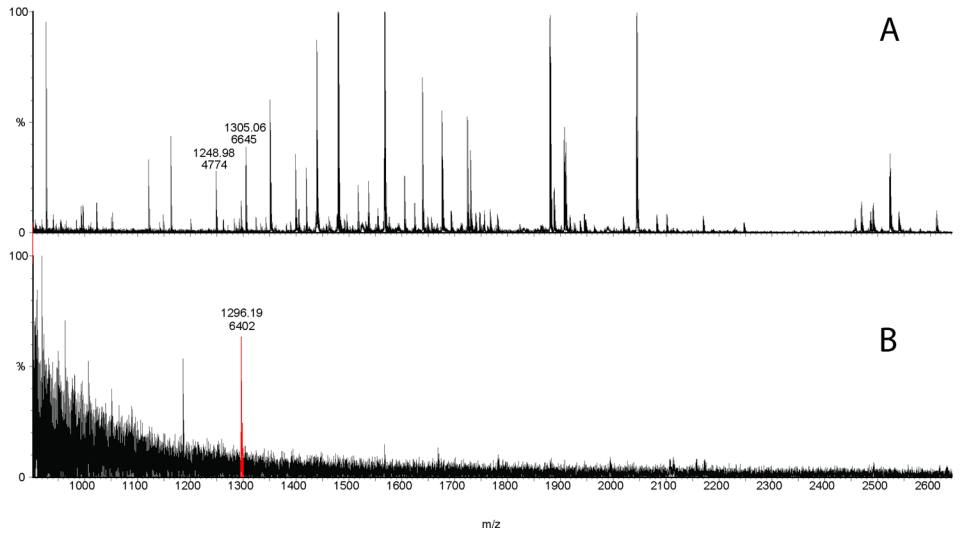

FIG. 5. Immunocapture from a complex sample. Top spectra (A) results after a direct RP-SPE sample preparation, i.e., no immunocapture (reference sample). Spectra (B) results after acoustic trapping iMALDI analysis of a 15 μl sample containing 1 nM Ang I in 200 fold excess of BSA digest (peptides). Note the lack of unspecific binding observed in spectra B.

Assay characteristics

The binding capacity for the acoustic system is determined by the bead type, the number of trapped beads and the antibody coverage. In the conducted experiments, the antibody was coupled to 2.8 μm magnetic Dynal-beads, as to allow direct comparison with a standard magnetic bead sample preparation protocol. The number of beads in a cluster was measured by forming and releasing clusters into a counting chamber. This was done for five clusters resulting in a measured trapping capacity of 580 000 beads with a standard deviation of $\pm$8.5%. The capability of trapping a defined amount of beads in the acoustic protocol can provide improved assay precision, as the variations due in bead volume and bead losses during washes and transfers in magnetic activated protocols are avoided. Since MALDI MS has relatively large natural signal intensity variation (30-50%), it is not possible to directly use signal intensity to measure reproducibility. A more relevant measurement is to measure the variation of the isotope ratio, this was done analyzing 9 consecutive plasma samples spiked with Ang 1 and its heavy isotope

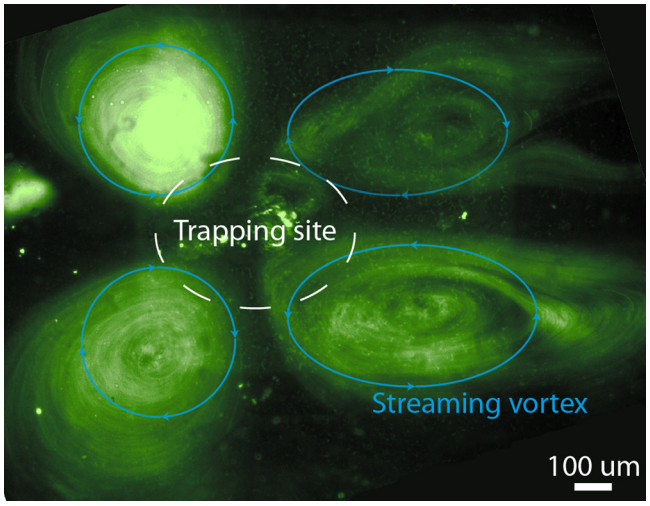

FIG. 6. Fluorescent image of the acoustic streaming phenomena visualized using 230 nm tracer particles. Dashed line shows trapping site and arrows the streaming vortexes.

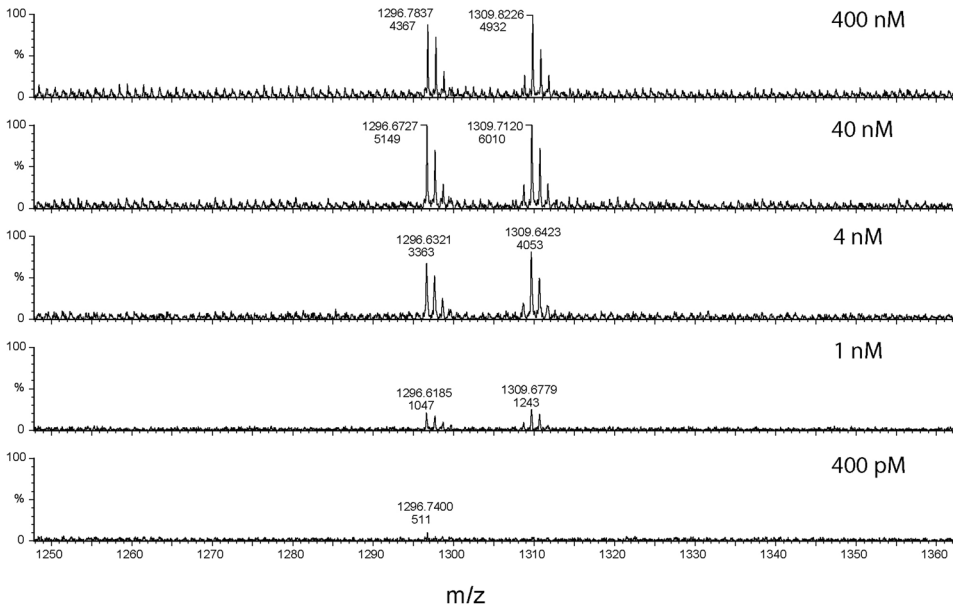

FIG. 7. Ang I (1296 Da) spiked in 1:10 human plasma together with a standard isotope of Ang I (1309 Da) at 8:10 ratio and falling concentration from 400 nM to 400 pM. It can be noted that the intensity increase levels out when processing concentrations higher than 4 nM. Also at the lowest analyzed concentration no isotope was added.

at concentrations 400-4 nM, which resulted in a CV of 4%.[59] During analysis of Angiotensin I and its heavy isotope at a fixed ratio of 10:8, using concentrations ranging from 400–0.4 nM, it was found that the observed signal intensity did not significantly increase after going to higher concentration than 4 nM, Figure 7. This indicates that the maximum capacity of the trapped bead cluster is approximately 40 fmol (4 nM × 10 μl). More importantly, it should be noted that in order to get similar results from the standard magnetic bead sample preparation protocol approximately 4–5 times more beads was needed. This is due to the difficulties associated with handling very small amounts of beads, i.e., bead losses during pipetting and transfer. The fact that acoustic trapping can be implemented using $^1/_4$ of the bead amount implies a corresponding cost reduction for the assay. As the cost of effective antibodies can be substantial a 75% reduction in the consumption is of large importance if an assay is to be applied in a clinical setting.

When the acoustic immuno-capture protocol was applied to perform immunocapture from a plasma sample, a sensitivity of 400 pM for a sample volume of 10 μl was obtained. In order to use the proposed platform in a clinical setting, the sensitivity requirement for the assay has to be reached. Angiotensin I is generated in plasma at a rate of 0.15–100 nM/h[61] that in order to ensure detection at the lower level approximately 25 μl of sample needs to be processed.

CONCLUSIONS

A new method for bead-based iMALDI-MS assays that utilize capillary acoustic trapping and microfluidic ISET based RP-SPE has been developed and provides some major advantages over current standard protocols.

The efficient washing that minimizes unspecific binding and enables removal of detergents. This also allows the use of detergents in the early stages of the experiment, e.g., for bead or sample solubilization, without interfering with the final iMALDI-MS read-out.

The trapping capillary is shown to provide the ability to capture a reproducible number of beads and facilitates the use of very small bead amounts. Furthermore, the microfluidic format in combination with the acoustic streaming enables reduced incubation time. This allows for reductions in assay time and cost.

The washing step is fast compared to the standard method where the beads have to be "pelleted" between each washing step. This greatly simplifies automation and few other current

techniques allow reproducible handling of such small bead amounts (500 000–600 000 beads). Further, the ability to minimize sample and bead losses provides high sensitivity in the final read-out.

In the presented work, magnetic Dynabeads were used, but the acoustic trapping is generic and can allow use of any bead type as long as the acoustophysical properties of the beads allow for acoustic trapping. Future work in our laboratory is focused on introducing additional sample processing capabilities, automation, and application of the technique to clinical samples.

ACKNOWLEDGMENTS

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